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Generation of transgenic Xist-BglG Halo ES cell line

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An abbreviated version of this protocol was published in Science in Jun 2021

Time-resolved structured illumination microscopy reveals key principles of Xist RNA spreading

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Detailed protocol

Generation of transgenic Xist-BglG Halo ES cell line

A plasmid vector encoding doxycycline inducible Xist containing an array of 18 Bgl-stem-loops within Exon 7, previously generated in the lab (Moindrot et al. 2015), was used to generate the autosomal Xist transgene cell line. Cells were additionally transfected with a construct encoding doxycycline inducible BglG-Halo protein, this vector encoding doxycycline inducible BglG-Halo fusion protein was generated by Gibson assembly using the Gibson Assembly® Master Mix (NEB) from different plasmids previously made in the lab containing sequences encoding for the doxycycline inducible promoter, the HaloTag and the BglG protein. The resulting vector also included a Neomycin resistance cassette for selection of clones with successful integration of the construct. Effective cloning of the vector was confirmed by Sanger Sequencing.

5 µg of the transgenic Xist construct and 2.5 µg of the BglG-Halo construct was transfected using TransIT-LT1 transfection reagent (Mirusbio) according to the manufacturer's instructions into C57BL/6JJcl x 129/SvJcl (P4D7) XY mESCs. 24hrs later, cells were passaged to 90 mm gelatine-coated Petri dishes with feeders and grown in medium containing 200 µg/ml neomycin (Geneticin® (G-418); ThermoFisher) for 10 days. Clones were picked and gDNA extracted, before successful insertion of the BglG-Halo sequence was screened for by PCR.

For PCR screening, cells were washed with PBS and snap frozen on dry ice. Next, they were incubated with squishing buffer containing 10 mM TrisHCl, pH 8, 1 mM EDTA, 25 mM NaCl and 200 µg/ml fresh Proteinase K at 65 °C for 30 min, before the Proteinase K was inactivated at 95 °C for 2 min. The extracted gDNA was used as template DNA for PCR analysis with the Mastercycler nexus (Eppendorf). PCR screening of the Bgl-stem-loop inserts and the BglG-Halo was not successful when using Taq DNA polymerase. Therefore, Velocity polymerase (Bioline) was used instead. Here, 1 µl of template DNA was incubated with 0.4 µl 20 µM forward and reverse primer each (Table 2), 0.4 µl Velocity polymerase, 4 µl 5x HiFi reaction buffer, 0.4 µl 10 mM dNTPs, 0.6 µl DMSO (final concentration of 3%) and 12.8 µl nuclease free water for a total reaction volume of 20 µl. Initial denaturation at 98 °C for 2 min was followed by 35 cycles of denaturation at 98 °C (30 s), annealing at 56 °C (30 s) and extension at 72 °C (30 s), with a final extension of 10 min at 72 °C. All PCR products were run on 1% EtBr Agarose gel at 110 V for 1 h together with the 1 kb+ ladder from New England Biolabs, and visualised with UV-light.

Autosomal transgenic Xist expression was assessed by Xist RNA FISH and subsequent widefield fluorescence microscopy with an inverted fluorescence Axio Observer Z.1 microscope (Zeiss). Clones with confirmed Xist expression were selected for HaloTag ligand staining and 3D-SIM to confirm BglG-Halo expression and efficient labelling of autosomal transgenic BglG-Halo- Xist. The final clone to be taken forward for analysis was selected based on suitable BglG-Halo expression levels that allowed for the best ratio of signal from BglG-Halo- Xist to unbound BglG-Halo. This was followed by characterisation of the newly autosomal transgene cells by determination of induction efficiency by Xist RNA FISH, combined HaloTag staining and Xist RNA FISH, and chromatin RNA sequencing, which allowed for the determination of the insertion site of autosomal transgenic Xist on chromosome 15.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Coker, H. , Schermelleh, L. and Brockdorff, N. (2023). Generation of transgenic Xist-BglG Halo ES cell line. Bio-protocol Preprint. bio-protocol.org/prep2255.
2. Rodermund, L., Coker, H., Oldenkamp, R., Wei, G., Bowness, J., Rajkumar, B., Nesterova, T., Pinto, D. M. S., Schermelleh, L. and Brockdorff, N. (2021). Time-resolved structured illumination microscopy reveals key principles of Xist RNA spreading. Science 372(6547). DOI: 10.1126/science.abe7500